Temperature Coefficients of Enzymic Activity and the Heat Destruction of Pancreatic and Malt Amylases

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University.

By

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To My Father and Mother

ACKNOWLEDGMENT

It is a great pleasure to acknowledge my gratitude to Professor H. C. Sherman for the initiation of this work and for his constant advice and supervision in its execution.

INTRODUCTION

There are in the literature many references to the action of heat on enzymic processes. In general these are not specific, and where they are they deal, as a rule, with one isolated point such as the temperature of optimum activity or that of destruction. Not much reliance can be placed on the figures given in some of the earlier work (1, 2), as at that time very little was known as to the conditions for the optimum activity of enzymes. The effect of heat on enzymic activity through a wide range of temperature has been little studied. Since enzymes are, in general, unstable substances, the effect of heat upon any reaction catalyzed by them is made up of a two-fold effect. Euler (3) points out that a rise in temperature first accelerates the chemical velocity and second, inactivates the enzyme. Thus in plotting curves of the activity of an enzyme with increase in temperature the curve always tends to rise to a maximum. Sorenson (4) says: "Therefore, the curve of the temperature should be considered as if it were two entirely different curves, namely, a curve of the real temperature effect which increases in proportion to the temperature elevation, not only below but probably above the optimum temperature, and on the other side a curve of the destruction of the enzyme." The maximum of this temperature curve varies according to the enzyme employed, the acidity, concentration of the substrate, presence of salts, type of solvent, etc. Much of the confusion arising from apparently conflicting data in the literature is due to the lack of fulfilling the conditions of optimum activity of the enzyme, especially in regard to hydrogen ion concentration and activating salts, the latter being very essential in the case of the amylases. Much of the work of various investigators cannot be compared quantitatively because of failure to insure the optimum hydrogen ion concentration. At any other hydrogen ion concentration the enzyme is less active and also, according to Lüers and Lorinser (15), less stable. This important condition was established for the temperature of 40° for pancreatic and malt amylases and "taka-diastase" by Sherman, Thomas and Baldwin (5).

It has been stated as a general rule that for every 10°C. rise in temperature, the velocity of a chemical reaction increased two to three times. Harcourt and Esson (6) found for the liberation of iodine from HI by H_2O_2 that between 0° and 50° the velocity was about doubled for every 10° rise. Hudson and Paine (7), working with invertase, found the coefficient of destruction by acids and

alkalies was between 2 and 3 for every 10° for the interval 0-45°. Auld (8) working with amygdalin-emulsin showed a temperature coefficient of 2 for every 10° between 15-35°C. Vernon (9) using starch and an amylase found that the velocity of hydrolysis was twice as great at 30° as at 20°. Kendall and Sherman (10) give 2 as the approximate temperature coefficient of pancreatic amylase for 10° between 20° and 40°.

It appears that with enzyme reactions, within the range of temperature where destruction of the enzyme plays as small a part as possible, that the temperature coefficient of the velocity of the reaction is of the same order of magnitude as those of other chemical processes. In the light of previous work it appeared that it might be of interest to study the action of temperature not only on the speed of hydrolysis but also on the rate of inactivation of the common amylolytic enzymes. Pancreatic and malt amylases were chosen for this investigation.

MATERIALS AND APPARATUS

The starch used in this work was Merck's "Soluble starch according to Lintner," washed nine times with distilled water and six times with triply distilled water. Moisture and acidity were determined, the latter by titration with 0.01 N NaOH using rosolic acid as indicator.

Ordinary distilled water was twice redistilled, first over alkaline permanganate solution, then over very dilute solution of orthophosphoric acid, condensing the distillate in a block tin condenser. This product was always used for making up starch and stock solutions and for the final rinsing of all glass ware.

The primary and secondary phosphates used as buffers were purified according to the unpublished methods of Beans and Kiehl. The NaCl used was twice recrystallized from triply distilled water and dried at 120°C.

The two enzyme preparations were commercial products: Parke Davis pancreatin, laboratory sample No. 9, and a barley malt obtained from the Froedtert Malting Co. The malt extract was prepared by grinding 100 grams of malt and extracting with 250cc. of triply distilled water at ice box temperature for two hours. The extract was then filtered and kept in the refrigerator in a steamed non-sol bottle. The total solids and reducing substances present in the extract were determined for each new preparation. This stock solution of malt extract was tested from day to day and did not change in activity during the time required to establish the points on the curves for that enzyme. The pancreatin was prepared fresh for each day's experiment and was made up by weighing out 50 mg. of the dry substance and making up to a definite volume with a cold solution of phosphate of the required concentra-

tion. The power of these preparations was determined according to the method of Sherman, Kendall and Clarke (11). The pancreatin had an average power of 300 on their scale and the malt extract a power of 48.

For thermostats 5-gallon iron cans, air stirred, were used, the temperature being maintained by means of an Ostwald mercury toluene regulator which held each bath at $\pm$ 0.15°C.

METHOD.

Since the object of this work was not only to determine the relative velocity of starch hydrolysis with increase in temperature but also to determine the rate of inactivation of the enzyme in the absence of substrate, the ordinary procedures had to be modified to a slight extent.

The following procedure was based on the work of Sherman and Walker (12).

For the direct hydrolysis, 6 grams of starch, on the dry basis, is weighed out and added to about 500cc. of triply distilled water at the boiling point. The mixture is boiled for two minutes, cooled and enough 0.01 N NaOH added to bring to neutrality, generally from 4 to 5cc., then 30cc. M NaCl and 15cc. of 0.02 M Na_2HPO_4 are added and enough triply distilled water to make the final volume 600cc.; this concentration of phosphate brings the hydrogen ion concentration to the optimum, which is 10^{-7} for pancreatic amylase.* This is thoroughly mixed and poured into 100cc. measuring cylinders which are then immersed in the thermostat at the temperature at which the digestion is to be carried out. 50 mg. of the enzyme preparation, in the case of pancreatin, is then weighed out and made into a smooth paste with a drop of cold water containing the same concentration of NaCl and Na_2HPO_4 as the digestion mixture. This is then washed into a 100cc. volumetric flask with the cold water salt solution, made up to the mark and kept at 10° or colder till used. Varying amounts of this solution, depending on the activity of the enzyme, were pipetted out in an accurately calibrated 1cc. pipette into dry 200cc. erlemeyer flasks and the starch mixture in the cylinders poured into the flasks at 15 second intervals and the flasks immersed in the bath and digested for one-half hour. At the expiration of this period 50cc. of Fehling's solution is added to each flask at 15 second intervals in the same order in which the starch was poured on to the enzyme. The flasks are then immersed in a boiling water bath and held at the boiling point for 15 minutes, then rapidly filtered through gooch crucibles, washed with hot water, alcohol and ether, dried at 100°C. for 20 minutes and the cuprous

* All measurements of hydrogen ion concentration were made at room temperature (about 22°). Statements regarding optimum are based on tests of activity in 30-minute digestions at 40°, and, therefore, may not represent the literal optimum for other times and temperatures.

oxide weighed; and the maltose calculated from Defren's table. In the case of malt extract the procedure is identical except that 2.5cc. of the extract was made up to 100cc. with cold triply distilled water without any salts; the starch mixture after neutralization is brought to the optimum hydrogen ion concentration of $10^{-4.5}$ by the addition of 18cc. of M NaH_2PO_4; since Sherman and Walker (13) found that for a 1% starch 3cc. of M NaH_2PO_4 per gram of dry starch was sufficient to bring the hydrogen ion concentration to the optimum for malt amylase. The hydrogen ion concentration of these solutions was determined both colormetrically and electrometrically. (See footnote on preceding page.)

The temperatures chosen were 20° to 70° by 10° intervals. As a rule 4 sets were run at the lower and one at 10° higher at one time, thus each new run overlapped the previous work and was a constant check on the technique and the activity of the enzyme. Blank experiments were made not only on the enzyme preparations, but also on the starch and reagents with each set. The results obtained from these sets gave the curve for the normal hydrolysis of the starch from 20° to 70°, the enzyme being almost completely inactivated at the latter temperature. Thus the curve represents the apparent activity of the enzyme at 10° intervals, both time and buffer concentration being held constant.

In order to study the direct effect of heat on the enzyme it is necessary to work in the absence of substrate as the presence of starch appears to exert a protective action. The modified procedure is as follows: 6 grams of dry starch is made up as before but with only one-half the triply distilled water and salts as were previously used, the final volume of the starch being 300cc. To 275cc. one-half the salts necessary for 600cc. were added and the volume made up to 300cc. Thus both the water and the starch dispersion contained the same concentration of activating salts and also the same relative amount as if they had been made up to a final volume of 600cc. The water salt solution is divided into 50cc. portions, put into test tubes and immersed in the bath. After coming to temperature they were poured on to the enzyme preparation at 15 second intervals and these enzyme solutions then placed in the thermostats and allowed to remain for 30 minutes. At the end of this period, the starch, previously brought to the same temperatures was added to the enzyme water mixture at 15 second intervals, and in the same order as the water salt solution, and this mixture allowed to digest for 30 minutes, thus giving a basis of comparison for every temperature interval up to the point where the enzyme was destroyed. The method of overlapping sets from day to day was followed here in order to guard against any inaccuracy of the method or deterioration of the enzyme. The 30 minute period of destructive heating, was, at the higher temperatures shortened to 15, 10 and 5 minutes in order to follow the rapid rate of destruction of the enzyme which was found to occur.

RESULTS

Since this investigation covered a wide range of temperatures it was necessary, at 20° and 30°, to increase the quantity of enzyme used in order to obtain enough cuprous oxide to weigh, while at the higher temperatures the activity of the preparations was so increased that much smaller amounts were used to prevent complete reduction of the Fehling's solution. In order to have a basis for the comparison of results and the plotting of curves it was decided to calculate the amounts of maltose formed for a fixed quantity of enzyme. The amount of enzyme chosen, which appeared to yield the most satisfactory figures for plotting, was taken as 0.1 mg. of the dry pancreatin or of the dry matter of the malt extract. By this method the data shown in Tables 1 and 2 were obtained. Table 1 gives the average amount of maltose obtained, per 0.1 mg. of pancreatin for each temperature, while Table 2 gives the same data for the malt extract. Using this as a basis Figures 1 and 2 were drawn, with temperatures as abscissae and amounts of maltose formed at these temperatures, per 0.1 mg. enzyme preparation, as ordinates. The upper curves show the velocity of hydrolysis of a one per cent starch solution by pancreatic and malt amylase at 10° intervals from 20° to 70°.

The lower curve, Figure 1, starts at 20° and is coincident with the other until 30° is reached and then falls below. This curve shows the activities of portions of the enzyme solution which had been heated in 50cc. of water salt solution for 30 minutes at the temperatures stated previous to allowing it to act on a one per cent starch solution. The lower curve, Figure 2, shows the same results for malt. The experimental results from which these curves were drawn are given in Tables 1 and 2 under the 30 minute heating period. Since in these destruction curves the enzyme is rapidly inactivated at the higher temperatures it was necessary to decrease the time of preliminary heating of the enzyme in the absence of substrate. This time was progressively decreased from 30 minutes to 15, 10 and 5 minutes. With these progressively smaller times the

TABLE 1.

Pancreatin No. 9.

Average of all results calculated to 0.1 mg. of enzyme

Temperatures	20°	30°	40°	50°	60°	70°
Without previous heating:						
Mg. Cu_2O	22.9	48.7	87.1	123.7	95.8	5.7
Mg. Maltose	18.3	39.1	70.5	100.2	77.5	4.5
After 30 minutes heating:						
Mg. Cu_2O	23.3	48.9	68.8	0.0		
Mg. Maltose	18.7	39.4	55.7	0.0		
After 15 minutes heating:						
Mg. Cu_2O	....	....	72.8	0.0		
Mg. Maltose	....	....	58.8	0.0		
After 5 minutes heating:						
Mg. Cu_2O	....	....	....	2.0		
Mg. Maltose	....	....	....	1.6		

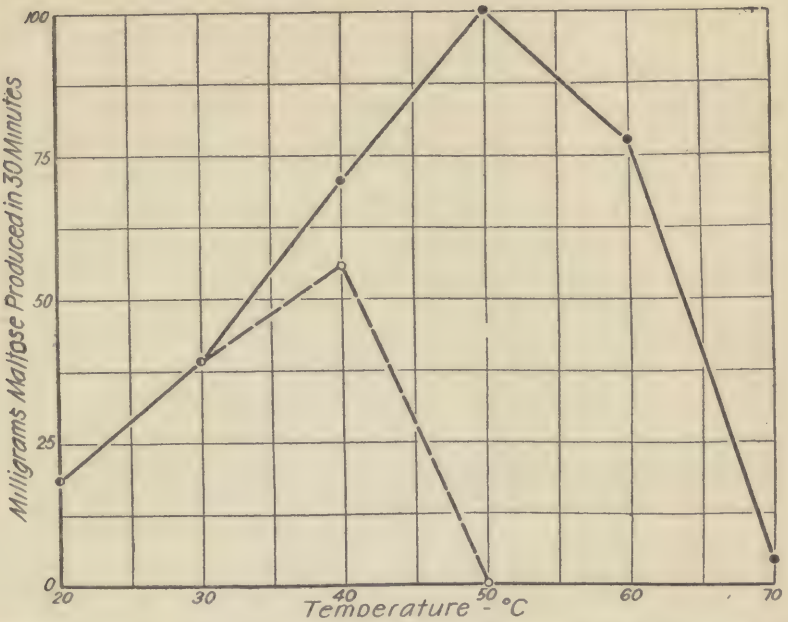

FIGURE 1.—The upper curve shows the effect of temperature on the hydrolysis of starch by pancreatic amylase. The lower curve shows the loss of activity of pancreatin, which previous to acting on starch has been subjected to 30 minutes' heating in water salt solution at the temperatures indicated. All curves drawn on the basis of the milligrams of maltose formed in 30 minutes per 0.1 milligram of dry pancreatin.

TABLE 2.

Malt Amylase

Average of all results calculated to 0.1 mg. of enzyme

Temperatures	20°	30°	40°	50°	60°	70°
Without previous heating:						
Mg. Cu_2O	4.5	8.4	13.6	17.9	13.7	0.7
Mg. Maltose	3.6	6.7	10.9	14.4	11.0	0.6
After 30 minutes heating:						
Mg. Cu_2O	4.4	7.5	10.6	13.5	0.5	0.0
Mg. Maltose	3.5	6.0	8.5	10.8	0.4	0.0
After 15 minutes heating:						
Mg. Cu_2O	....	....	....	14.3	2.0	
Mg. Maltose	....	....	....	11.5	1.6	
After 10 minutes heating:						
Mg. Cu_2O	....	....	....	....	3.6	
Mg. Maltose	....	....	....	....	3.0	
After 5 minutes heating:						
Mg. Cu_2O	....	....	....	....	6.8	
Mg. Maltose	....	....	....	....	5.5	

rates of destruction at the lower temperatures became unmeasurable and so no results are given for pancreatin, Table 1, below 40° after 15 minutes heating, and below 50° after 5 minutes heating. Likewise, for malt it became impossible to measure the destruction after

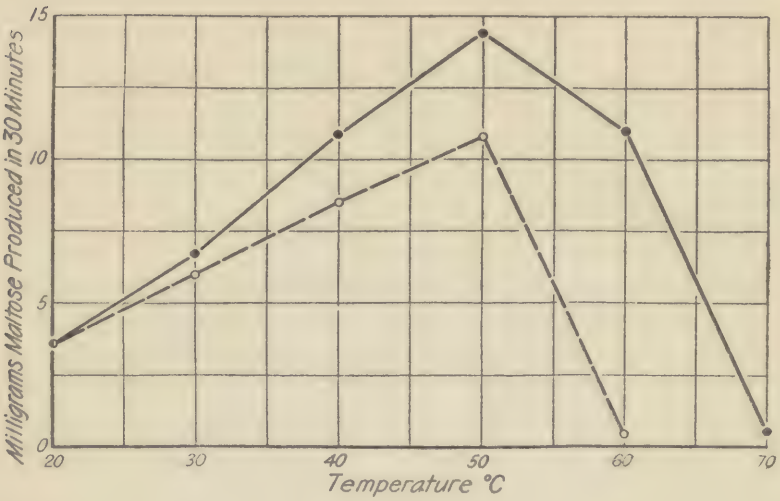

FIGURE 2.—The upper curve shows the effect of temperature on the hydrolysis of starch by malt amylase. The lower curve shows the loss of activity of malt, which previous to acting on starch has been subjected to 30 minutes' heating in water salt solution at the temperatures indicated. All curves drawn on the basis of the milligrams of maltose formed in 30 minutes per 0.1 milligram of the dry matter of the malt extract. The scale of these curves is five times that of Figure 1.

15 minutes heating below the temperature of 50°, Table 2, and after 10 minutes and 5 minutes below the temperature of 60°, Table 2. From the data in these tables the rate of destruction of pancreatic and malt amylases Figures 3 and 4, has been calculated. The method for obtaining the per cent destruction in these curves will be given under the discussion of results.

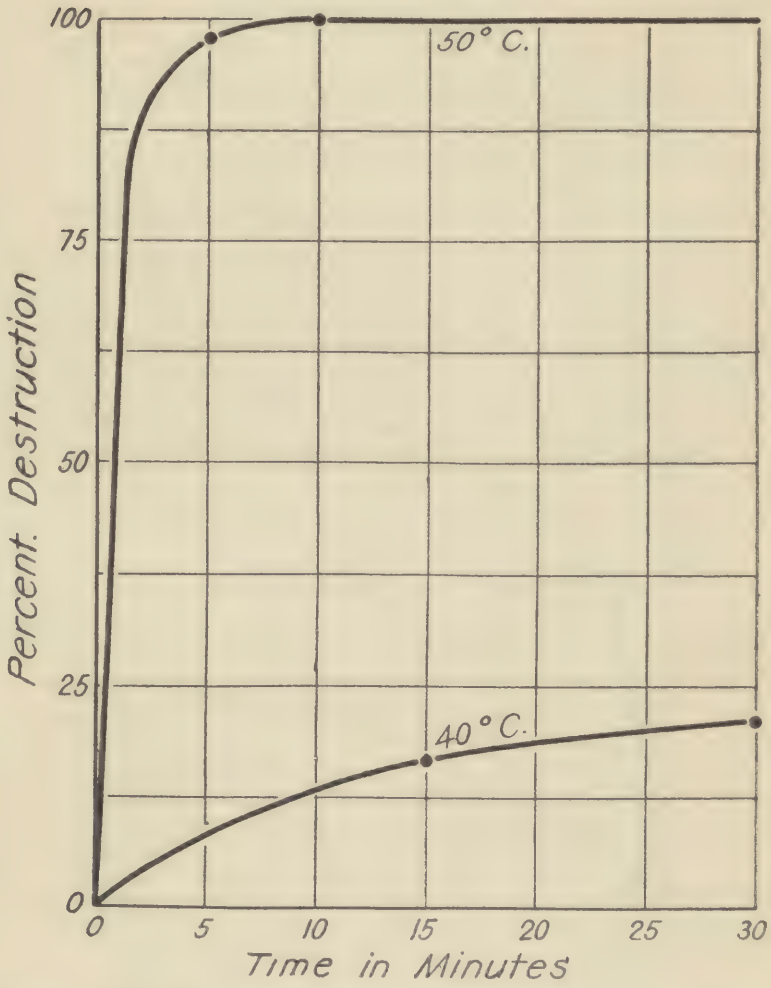

FIGURE 3.—The upper curve shows the rate of destruction of pancreatic amylase when heated in water salt solution at 50° previous to acting on starch. The lower curve shows the rate of inactivation under the same conditions at 40°.

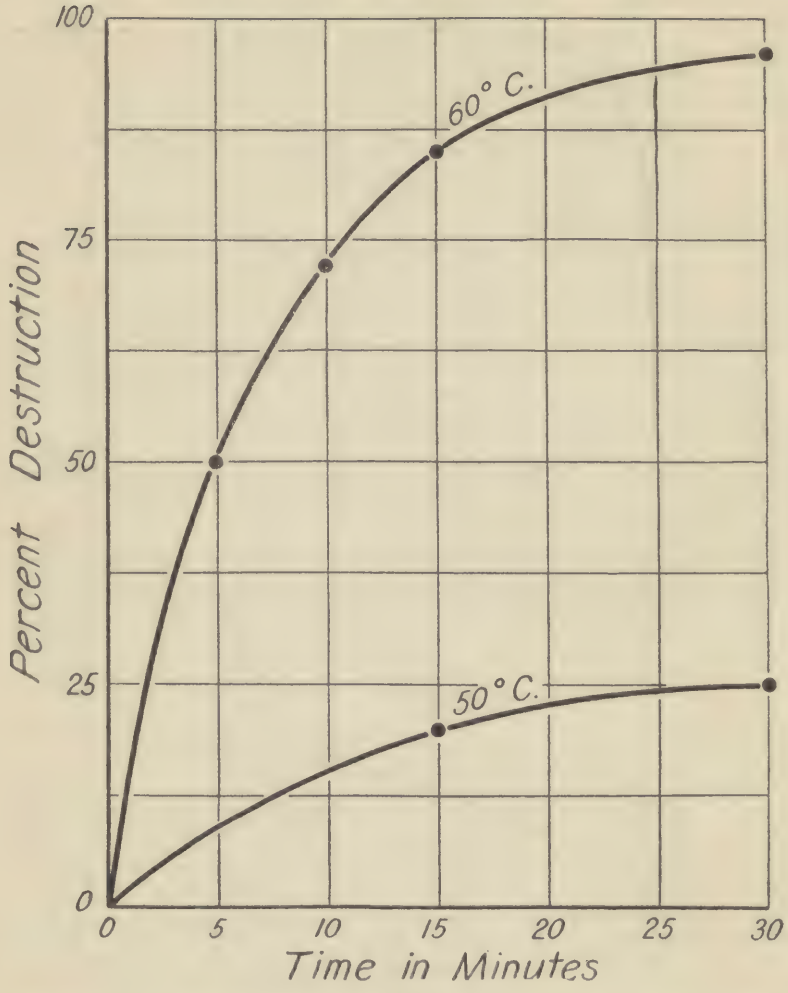

FIGURE 4.—The upper curve shows the rate of destruction of malt amylase when heated in water salt solution at 50° previous to acting on starch. The lower curve shows the rate of inactivation under the same conditions at 40°.

DISCUSSION OF RESULTS.

The first point worthy of note is the uniformity of the temperature velocity curves of both pancreatic and malt amylase. Though the enzyme preparations differ in activity the curves both rise to a maximum at 50° and drop rapidly to zero at 70°. Evidently the increase of chemical velocity is greater, up to 50° than the inactivation of the enzyme. Beyond 50°, however, the rate of inactivation increases to such an extent that it becomes the predominating factor and thus the curve drops rapidly. It is evident that the hydrolysis of starch by these two enzymes is similarly affected by temperature and that their stability in the presence of substrate is about the same.

The second point of interest is that of the heat destruction (lower curves, Figures 1 and 2), when the enzyme previous to its action on starch has been exposed to the effect of heat while in a water salt solution only. The divergence between the upper and lower curves shows the extent of this heat destruction in 30 minutes at each temperature. With pancreatic amylase the maximum activity lies at 40° and drops to zero at 50°, showing that within the period of 30 minutes, heating at 50° all the enzyme is destroyed. Malt amylase, however, continues to show increased activity up to 50° and is not entirely destroyed, even after 30 minutes heating at 60°. This indicates that malt amylase is much more stable than pancreatic amylase and this evidence is confirmed by unpublished data from this laboratory and work carried on by Ernstrom (14).

In order to follow this destruction more closely the time of the preliminary heating was reduced to 15, 10 and 5 minutes, as explained before. The amount of sugar produced in the hydrolysis (upper curves, Figures 1 and 2), at the particular temperature was called 100 per cent and that produced after different times of the preliminary destructive heating calculated to per cent and subtracted from 100 gives the destruction used in plotting curves 3 and 4. From this can be seen the relative velocity of destruction of pancreatin and malt. At 50° the pancreatin is about 97 per cent destroyed in 5 minutes while the malt has lost only 25 per cent of its original activity after one-half hour. At 60°, which is much above the point of destruction of pancreatin, the malt retains a slight activity even after 30 minutes.

From the data at hand it has not been found possible to use either the mono or bimolecular equations since the substitution of the data in these equations does not give a constant. Lüers and Losiner (15) and Ernstrom (14) found the mono and bimolecular equations inapplicable to enzyme inactivation curves. The Schultz law does not give a constant, though it is interesting to note that the empirical formula $k = \frac{X}{\sqrt[4]{t}}$ used by La Mer (16) to express the rate of destruction of vitamin C gives an approximate

constant. Since such a wide range of temperatures was used in the velocity temperature curves this necessitated a varying concentration of enzyme, and again it is found impossible to fit the results into any of the above mathematical expressions. However, we can arrive at an approximation of the velocity. At temperatures sufficiently low where the inactivation of the enzyme plays a small part, the ratio of the sugar formed by equal amounts of enzyme at the lower temperature compared to that formed at the next higher temperature will be a fair measure of the velocity of the action at these two temperatures. Thus for pancreatin in the interval 20°-30° the ratio is 2.1 and between 30° and 40° is 1.8, while for malt at the same points it is 1.87 and 1.62. From these results it would appear that the temperature coefficient of hydrolysis of starch by these two enzymes is about that of inorganic reactions. These results are also in accord with the data of other investigators. The velocity of the inactivation of the enzyme is, however, a good deal more rapid than this; pancreatin, for instance, under the conditions of these experiments is destroyed 30 times as rapidly at 50° as at 40° if we compare times of equal destruction. In a like manner malt shows a rate of destruction of about 15 times as great at 60° as at 50°. It is of interest to compare these values with those which La Mer, Campbell and Sherman (16) determined for the heat destruction of vitamin C. Working at 60°, 80° and 100°C. they found the temperature coefficient per ten degrees to be 1.23 to 1.12. The rate of destruction of pancreatic amylase can only be roughly compared, since it is rapidly inactivated at 50°, a temperature 10° lower than any given in La Mer's work. However, extrapolating from his data we can assume 20 per cent destruction of the vitamin in four hours. The heat destruction of pancreatic amylase would then have a rate somewhere in the neighborhood of 500 times as great as that of vitamin C at this temperature. At 60° his curves show a destruction of 35 per cent of vitamin C in 4 hours. The rate of inactivation of malt amylase at this temperature is about 100 times as great.

It will be noticed that the lower or destruction curve, Figure 1, for pancreatin reaches a maximum at 40° and drops rapidly to zero at 50°, while in the presence of starch the maximum point not only shows a greater amount of maltose formed, but also lies at a temperature of 10° higher; thus showing the powerful protective action of the starch which might be interpreted as due to the formation of some sort of starch-enzyme complex. Malt amylase shows a greater stability toward heat, since the maximum of the lower curve, Figure 2, lies at 50° instead of 40°. It is also evident that the presence of starch has less protective action than in the case of pancreatin. That this is due to the lower activity of the malt and the consequent presence of impurities that may act as protecting agents is hardly a valid assumption in the light of preliminary experiments carried on with an old preparation of pancreatin of about

one-third the power of sample No. 9. This gave identical points of maxima on both the velocity and destruction curves. It would seem that malt amylase is inherently more stable than pancreatic amylase.

There are two possible explanations:

1. The inactivation of the enzyme may be a hydrolysis. The concentration of the enzyme is at all times very low. Even with the commercial preparations worked with there is never more than 2 milligrams of the enzyme preparation in the 50cc. of water used in the destruction tests. It was at first thought that possibly the dilution was a factor of importance and a series of tests were run in which the enzyme concentration was decreased to one-half, and one-fourth, but no change in the rate of inactivation could be noticed. This was interpreted to mean that the dilution was already so high that hydrolysis was at a maximum. Due to the activity of the sample and the rapid rate of starch cleavage it was impossible to increase the concentration of the enzyme beyond the point where reducing sugar corresponding to 250 to 300 mg. of cuprous oxide was formed in the time of the experiment, and this amount compared to the volume of water was insignificant. As was pointed out the destruction of pancreatic amylase at 50° in these experiments was much more rapid than the ordinary hydrolytic reactions which have been studied previously—Arrhenius (18).

2. If the amylases are protein in nature it is possible that their inactivation might be due to heat coagulation, which, with most proteins, occurs between 50° and 70°. Sherman and Schlesinger (17 and 19) working with a purified pancreatic amylase and malt amylase, found a faint opalescence in their solutions at 50°, with increasing coagulation up to 70°, at which point, after filtering, no further precipitation would occur, even at high temperatures. It is quite possible that the previous formation of this coagulum would, on the later addition of starch, prevent any action of the enzyme. On the other hand, since enzyme action is probably a surface reaction, in the presence of the starch the enzyme at coagulation temperatures would be precipitated on the starch molecule, and though its activity would probably be less, still the action would continue beyond the point where it could react with the substrate after a preliminary heating that brought about coagulation. This theory is in accord with the evidence as shown by the curves.

From the data at hand neither theory is susceptible of proof. Hydrolysis and coagulation of a protein is non-reversable. Ernstrom (14) found that the activity of malt and salivary amylases after destruction by heat could not be regenerated. The work of Sherman, Walker, Caldwell and Naylor (20, 21, 22, 23) on the protective action of certain amino acids on the amylases supports the theory that hydrolysis plays an important role in the heat destruction of the enzymes.

DISCUSSION OF ERRORS.

The errors involved in this work can be divided into those of apparatus, method and personal.

All volumetric apparatus was standardized and the error involved here is negligible. The method of temperature control of the thermostat is only accurate within $\pm$ 0.15° which in 10° intervals makes possible an error of 3 per cent. But since during the 30 minute period of heating the bath is not always above or below, but remains for considerable periods on the mark this error is appreciably less. At 60° and 70°, however, the lag of the bath may bring this error up to 3 per cent. All thermometers were standardized against a Bureau of Standards thermometer calibrated in tenths, and corrections were applied for emergent stem and the error of reading would fall well within 0.5 per cent. Summing up the errors involved in temperature control they fall well within 4.0 per cent.

Concerning errors of method the results show that they fall beyond the limit set in the case of apparatus. There are a good many factors to be taken into account. The instability of enzymes in general makes it difficult to get close checks from day to day. Precautions as to the purity of reagents, cleaning of glassware, fumes in the room, light acting on enzyme solutions, traces of saliva in pipetes, etc., make it impossible to work with the same degree of accuracy expected in ordinary quantitative analysis. Using Defren's method of sugar estimation and pure sugars the error between duplicate determinations is low, something of about the order of 0.5 per cent. In estimating the reduction products formed by the action of the amylases on starch this error is greater, in general it has been found possible in this laboratory to obtain results that check within 3 or 4 milligrams of cuprous oxide. This, in 250 milligrams, might amount to nearly 2 per cent, but it must be remembered that this limiting value was attained in most cases on work carried out under the favorable conditions of constant and accurately controlled temperature, with fixed amounts of enzyme. In the work recorded here the temperature varied through a wide range and it was necessary to increase the proportion of enzyme at low temperatures in order to get sufficient cuprous oxide formed. In the velocity curves the results show that an accuracy of about 6.0 per cent is easily possible, and almost all of the points in the curve are within 4.0 per cent or less. The direct weighing of the cuprous oxide instead of determining the copper electrolytically introduces no appreciable error as Quisumbing and Thomas (24) found that the two agreed closely.

In timing the periods of digestion or destruction a stop watch was used, and as a rule 15 second intervals were allowed for manipulative details and were found ample to permit of close control of the time factor. A difference of $\pm$5 seconds in the interval of 30

minutes was therefore only about 0.6 per cent and was negligible, but in the short destruction times a difference of ± 5 seconds would bring the possible error up to 3.0 per cent.

At high temperatures the introduction of a cold 200cc. Erlenmeyer flask into the thermostat not only tended to lower the temperature of the bath but the flask itself took an appreciable length of time to come to equilibrium, and this introduced an error of unknown magnitude, but since all procedures were standardized throughout the work the results are comparable. Thus the curve for the destruction of pancreatin at 50°, Figure 3, is probably less definitely established than that of malt at 60°, due to the difficulties of getting concordant results at such short time intervals, but this does not invalidate the general results which show the greater instability of this enzyme compared to malt. For this reason no great emphasis is placed on the fact that in Figure 2 the destruction curve of malt breaks away from the upper curve at 30°, while in Figure 1 pancreatin, which is more unstable, does not do so. This deviation with malt is a little beyond the average experimental error, and though all the results point to there being an appreciable destruction at 30°, still it is felt that the methods are as yet not sufficiently exact to permit of absolute certainty regarding this result.

It is believed that owing to the well-known sensitiveness of the amylases to hydrogen ion concentration and the presence of salts, that the addition of the necessary amounts of the reagents to the starch before measuring out into the 100cc. cylinders cuts down one possibility of error.

The personal errors in this work would be largely errors of manipulation, such as varying the time of pouring the starch into the enzyme, consistently reading volumes, high or low, etc. The concordance of experimental results themselves, are the best check on such inaccuracies.

Each point on the curves is the result of from five to ten separate determinations, and the results as given are believed to be within an error of 7.0 per cent at the most, and generally the results are within 4.0 per cent or less.

CONCLUSIONS.

1. The rates of starch hydrolysis by pancreatic and malt amylases used in the forms of good grades of commercial pancreatin and malt have been determined for the temperature range of 20° to 70°C. At temperatures below the point where destruction of the enzyme plays an important role the rate of hydrolysis is about doubled for every ten degrees rise in temperature.

2. The temperature and rate of destruction of these enzymes in water salt solution has been determined and malt amylase is found to be much more stable than pancreatic, the latter being com-

pletely destroyed in 30 minutes heating at 50°, while malt still shows a trace of activity after 30 minutes at 60°C; the enzyme of pancreatin being destroyed approximately 30 times as fast at 50° as is that of malt.

3. The curves obtained show graphically the great protective influence of the substrate on these enzymes at high temperatures.

4. The results obtained for the rate of destruction of these enzymes show a wide divergence from those giving the rates of destruction of vitamin C, and make it appear doubtful that any advantage is to be gained by classing vitamins as enzymes as has been recently suggested.

5. The results obtained support the view that the heat destruction of the enzyme may be a process of the nature of the coagulation of a protein.

6. From the similarity of the curves it appears probable that the two enzymes studied act upon starch in much the same manner; and the results give added evidence for the belief that the action of these enzymes on the starch involves the formation of some sort of starch-enzyme complex.

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